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# Hyperbranched polyphosphates and their biomedical applications



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# Hyperbranched polyphosphates: synthesis, functionalization and biomedical applications

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## Key Learning Points

(1) Outline why biocompatible and biodegradable hyperbranched polymers are important for biomedical applications.

(2) Hyperbranched polyphosphates open a new window for the development of biomedical hyperbranched polymers.

(3) The topologic structures and functionalization of hyperbranched polyphosphates can be controlled via adjusting the side group of cyclic phosphate monomers.

(4) Showcase the promising utility of hyperbranched polyphosphates in biomedicine, especially for drug delivery applications.

(5) Summarize the progress in this emerging field and define challenges for the future.

**Abstract**

Hyperbranched polyphosphates (HBPPs) are newly emerged polymeric biomaterials with repeating phosphate bonds in the highly branched framework over the past 5 years. Due to the integration of the advantages of both hyperbranched polymers and polyphosphates, HBPPs are versatile in chemical structure, flexible in physicochemical properties, water soluble, biocompatible and biodegradable in biological feature. On the basis of their excellent water solubility, biocompatibility, biodegradability and potential functionalization as well as simple preparation in one-pot, HBPPs are fascinated in biomedical applications, especially for drug delivery. In this Tutorial Review, the recent advances of HBPPs have been summarized. HBPPs with different topological structures and various functionalities have been synthesized via adjusting the side group of cyclic phosphate monomers, which have shown promising biomedical applications, for example, using as a macromolecular anticancer agent and constructing advanced drug delivery systems including site-specific delivery systems, self-delivery systems, and stimuli-responsive delivery systems. Such progresses may promote the further development of interdiscipline researches between polymer chemistry, material science and biomedicine.

## 1 Introduction

The concept of 'highly branched polymers' was first mentioned in 1952 during the theoretical study of condensation of $AB_n$ ($n \geq 2$) monomers that forms polydisperse highly branched architectures with many terminal groups. However, the synthesis of dendritic polymers including dendrimers and hyperbranched polymers (HBPs) has been achieved until the end of 1980s, which have been considered as the fourth major polymer architecture following the linear, branched, and crosslinked polymers. In contrast to dendrimers with perfectly branched and monodisperse structure but time-consuming stepwise synthesis and low yields, HBPs are often synthesized via one-pot protocol with rather irregular branched structure due to the random distribution of dendritic, linear and terminal units in their backbone. Fortunately, HBPs still have demonstrated several characteristics similar to dendrimers, such as a large number of terminal groups, three-dimensional (3D) globular structure, low viscosity of solution or melt, and good solubility. However, these unique properties cannot be reached by the corresponding linear analogues. Thus HBPs have aroused great interesting from the academic and industrial points of view, and a rapid progress has been made in this field. In the past two decades, great efforts have been made on syntheses, characterizations, functionalizations and industrial applications of HBPs, which have been summarized in several excellent reviews by Kakimoto,[1] Yan,[2] Voit[3] and Carlmark.[4] In recent years, HBPs have demonstrated great potential applications in functional materials and biomedicine, such as antibacterial/antifouling materials, cytomimetic chemistry, protein purification/detection/delivery, drug/gene delivery and

bioimaging. Progresses in these areas have also been highlighted in several distinctive review articles.[5-9] It's worth mentioning that to date only two commercial hyperbranched products mainly dominate this field, i.e., Perstorp "Boltorn" products of hyperbranched polyesters $H_x$ (x = 20, 30, 40) and HyperPolymers product of hyperbranched polyglycerols. Nevertheless, the non-biodegradability of hyperbranched polyether and the hydrophobicity of hyperbranched polyester impede their biomedical applications to some extent. Therefore, the design and synthesis of HBPs with good hydrophilicity, biocompatibility, and biodegradability simultaneously becomes a serious challenge to promote the development of polymeric biomaterials.

Polyphosphoesters are one kind of polymers with repeating phosphoester in the main chain. Functional/bioactive groups can be readily introduced into such polymers and their physicochemical properties can also be modified easily due to the multivalent property of phosphorus atom. According to the side group connected to the phosphorous atom, these polymers are classified into polyphosphate, polyphosphonate, polyphosphite, or polyphosphoramidate (**Figure 1**). To prepare polymer-inorganic hybrid materials and mimic biomineralization, Penczek et al pioneered the synthesis of polyphosphoesters at the end of 1970s.[10] Since then, several different approaches including ring-opening polymerizations (ROP),[11] polyaddition,[12] polycondensation,[13] transesterfication[14] and enzyme-catalyzed synthesis[15,16] have been reported for the synthesis of linear polyphosphoesters. Among them, polyphosphates represent an important family of biomaterials with excellent biocompatibility, biodegradability and similar structure to nucleic and teichoic acids.

Under physiological condition, polyphosphates can degrade into harmless low molecular weight species through hydrolysis or enzymatic digestion of phosphate bond. They also display good flexibility by the convenient functionalization of phosphorus, i.e., adjusting the pendant group of phosphorus. Therefore, the applications of polyphosphates have received great attention in biomedical fields, including controlled drug release, gene delivery and tissue engineering. Detailed references in this field are summarized in the reviews by Leong,[17] Chaubal[18] and Wang.[19] In recent years, the synthesis and functionalization of polyphosphates is still a popular topic and largely explored by many groups, such as Wang,[20] Wooley,[21,22] Wurm,[23] Momekov,[24] Ni,[25] Iwasaki,[26] Yang,[27] etc. These works are mainly focused on the design and preparation of functional polyphosphates with specific structures and unique properties that aim to improve the performance in controlled release of drugs/genes/proteins and scaffolds for tissue engineering. It is noted that that all of these works are limited to linear polyphosphates that will be excluded from this review, since this topic is extensive and contains sufficient material for individual reviews. Considering the property is usually determined by the structure of polymers, polyphosphates with various topological structures have been designed and prepared extensively to satisfy the requirement of biomedical application. Here we have specifically focused on the unique polyphosphates with hyperbranched structure, and hope to promote the development of hyperbranched polymers with good hydrophilicity, biocompatibility and biodegradability simultaneously for biomedical applications.

## 2 Hyperbranched polyphosphates (HBPPs)

HBPPs are one kind of biodegradable hyperbranched polymers with repeating phosphate bond in the branched backbone, which were initially synthesized to use as curing agent to flame retardant systems. No attention has been received for the biomedical applications of HBPPs until Yan's laboratory reported the synthesis of highly purified HBPPs with controllable structure and adjustable properties via self-condensing ring-opening polymerization (SCROP) in 2009.[28] Since then, various HBPPs with different topological structure and various functionality including amphiphilicity and specific stimuli-responsiveness have been synthesized by adjusting the side group of cyclic phosphate monomers (CPMs).

### 2.1 Synthesis of HBPPs

In the early years, HBPPs were mainly prepared by the "$A_2 + B_3$" approach that is a polycondensation method to form HBPs through the repetitive reaction between functional group A and B in difunctional monomer $A_2$ and trifunctional monomer $B_3$, respectively. Shi et al. reported an aromatic HBPP that was synthesized by the polycondensation of bisphenol-A ($A_2$) and phosphoryl trichloride ($B_3$).[29] No gelation was observed in the polycondensation process even if at the equal molar ratio of the functional group A and B. The DB of the resulting aromatic HBPP was 0.50 according to phosphorus nuclear magnetic resonance ($^{31}$P NMR) spectrum. Moreover, an epoxy-terminated HBPP was prepared by proton transfer polymerization of

1,4-butanediol ($A_2$) and triglycidylphosphate ($B_3$) with $Bu_4NCl$ as a catalyst to avoid crosslinking.[30] In addition, a series of nitrogen-containing HBPPs with many acrylate terminal groups were synthesized by the Michael addition reaction between piperazine and tri(acryloyloxyethyl) phosphate at different molar feed ratio of monomers.[31] The resulting HBPPs have high molecular weight and relative narrow polydispersity. Recently, Zhang et al. prepared a series of HBPPs with DBs over 0.87 from the polycondensation of 1,3,5-tris(2-hydroxyethyl)cyanuric acid and phosphorus oxychloride, and further studied their thermal degradation properties.[32] More recently, Zhan et al. successfully developed a kind of silicon-containing HBPPs through the Michael addition polymerization of (3-aminopropyl)trimethoxysilane with equimolar tri(acryloyloxyethyl) phosphate and investigated their flame retardancy properties.[33]

Our laboratory designed and prepared a new hydroxyl functionalized CPM via esterification of commercial available 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) with various diol molecules.[28] As a typical AB* inimer (i.e. initiator-monomer), CPM contains a reactive five-membered ring of phosphate and a self-initiating primary hydroxyl group. Therefore, HBPPs can be easily obtained through SCROP of CPM (**Figure 2**). Namely, the primary hydroxyl in one CMP molecule triggers the ring-opening reaction of the phosphate five-membered ring in another CMP molecule to produce a dimer with one phosphate five-membered ring and two primary hydroxyls. Further ring-opening reaction between a CMP and a dimer forms a trimer with a phosphate five-membered ring and three hydroxyls. Finally, HBPPs with high molecular weight are obtained by the repeating ring-opening reaction among the

formed species. The SCROP of CPMs can proceed smoothly in bulk at room temperature without catalysts. The resulting HBPPs are highly purified materials and especially suitable for biomedical applications. More important, the molecular weight of the resulting HBPPs can be controlled simply by adjusting the reaction temperature as well as the polymerization time. The DBs of the resulting HBPPs are around 0.5, indicating their highly branched structures. Commonly, HBPPs have good solubility in polar solvents such as water, alcohol, dimethylformamide and dimethylsulfoxide due to the existence of many polar phosphate units and terminal hydroxyl groups, but insoluble in chloroform, dichloromethane, tetrahydrofuran etc.

### 2.2 Functionalization of HBPPs

Until now, two different approaches have been developed to functionalize polyphosphates: (i) Conjugating functional or bioactive moieties to the reactive side groups of polyphosphates; (ii) Changing the backbone structure of polyphosphates by using diverse co-monomers. By facilely introducing various functional units into the side group of CPMs, a variety of HBPPs with different functions have been obtained through SCROP of the corresponding CPMs.

#### Hydrophilic HBPPs

Hydrophilic HBPPs can be synthesized by SCROP of the CPM with a pendent group containing a hydrophilic segment and a terminal hydroxyl group. For example, the CPMs with hydrophilic hydroxyethyl and hydroxyethoxyethyl side groups were

prepared by the esterification of COP with ethylene glycol and diethylene glycol respectively.[28,34] Then the hydrophilic hyperbranched poly(2-hydroxyethyl phospholane) (HPHP) and hyperbranched poly(2-hydroxyethoxy)ethyl phospholane) (HPHEP) were separately obtained by SCROP of corresponding 2-hydroxyethyl phospholane (HP) and (2-hydroxyethoxy)ethyl phospholane) (HEP) in bulk at room temperature. These HBPPs are highly water-soluble due to the existence of many hydrophilic phosphate units in branched framework and a plenty of terminal hydroxyl groups, which exhibit great potential for bio-conjugation.

**Amphiphilic HBPPs**

If some hydrophobic segments are introduced into the pendent group of CPMs, amphiphilic HBPPs can be achieved by SCROP of such CPMs. For instance, amphiphilic hyperbranched poly(5-hydroxypentyl phospholane) (HPHPP) was synthesized by using 5-hydroxypentyl phospholane (HPP) as monomer.[35] As an amphiphilic homopolymer with alternative hydrophobic pentyl units and hydrophilic phosphate segments in the highly branched framework, HPHPP can self-assemble into multi-core/shell micelles in aqueous media with diameter of several tens nanometers. This unique self-assembly behavior of HPHPP was attributed to its distinctive molecular structure. Clearly, the DB of HPHPP is around 0.5 so that almost half hydrophobic pentyl units located at the peripheral terminal units. Moreover, HPHPP has a very flexible branched backbone that can easily adapt the conformational change in the process of phase segregation. Thus both intra- and inter- molecular

microphase segregations may take place quickly. Due to the confined sizes of many hydrophobic and hydrophilic segments in this macromolecular precursor, the micelle is speculated with a multi-core/shell structure (**Figure 3**).[36] Namely, the micelle is consisted of many hydrophobic micro-domains (i.e. the aggregated region of pentyl units) which evenly distributed in the continuous hydrophilic phase (i.e. the aggregated region of polyphosphate segments). The self-assembled HPHPP micellar structure is further stabilized by the hydrogen bonds between -OH and -P=O groups. So far, most reported precursors for the self-assembly in selective solvents are amphiphilic linear/dendritic block copolymers. By using this unique amphiphilic HPHPP, the self-assembly of homopolymers has been realized, which enriches the research field of molecular self-assembly and also provides a charming pathway to construct drug carriers.

**Reduction-responsive HBPPs**

Reduction-responsive polymers, an important kind of environment-sensitive polymer, show great interesting in biomedical applications because of the existence of redox potential gradient *in vivo*. However, backbone reduction-responsive polymers with good biodegradability and biocompatibility are rarely reported. Recently, an attractive strategy has been reported to synthesize such polymers by SCROP of CPMs with a disulfide-containing pendent group.[36] Hyperbranched poly(2-(2-hydroxyethyl)-disulfanyl)ethyl phospholane) (HPHSP) was generated by using 2-(2-hydroxyethyl)-disulfanyl)ethyl phospholane (HSP) as monomer. It is worth mentioning that HPHSP

is an amphiphilic homopolymer with alternative hydrophilic phosphate and hydrophobic disulfide groups along the highly branched framework, which can self-assemble into multi-core/shell micelles in water with pretty narrow size distribution. The size of HPHSP micelles strongly depends on its molecular mass, which increases from 30 to 78 nm with the increase of molecular mass from $3.1\times10^3$ to $1.1\times10^4$ g $moL^{-1}$. Nile red-loaded HPHSP micelles in phosphate buffered saline (PBS) show significant decrease of the emission intensity of Nile red after the addition of dithiothreitol (DTT), suggesting the destabilization of micellar structure through the cleavage of disulfide bonds and subsequent release of dye molecules (**Figure 4**). Meanwhile, the size of HPHSP micelles decreases from 80 nm to 30 nm after 48 h incubation with 10 mM DTT while varies slightly without DTT. These indicate that HPHSP is stable under normal physiological environment, however, exhibits sensitive responsiveness under reductive conditions. The introduction of disulfide bond into the framework of hyperbranched polyphosphates provides a new way to prepare backbone reduction-responsive polymers with good biodegradability and biocompatibility, simultaneously.

**Oxidation-responsive HBPPs**

Similar to reduction-sensitive polymers, oxidation-responsive polymers are another important class of environment-responsive polymers and receive increasing attentions for biological applications, such as biosensor/biodetector and drug delivery.[37] However, the investigation of oxidation-responsive HBPs is very limited due to the

lack of efficient synthetic methods. On the basis of SCROP of CPMs, oxidation-responsive hyperbranched poly(2-(2-hydroxyethyl)selanyl)ethyl phospholane) (HPHSeP) has been synthesized from 2-(2-hydroxyethyl)selanyl)ethyl phospholane (HSeP) monomer.[35] Obviously, HPHSeP is also an amphiphilic homopolymer with alternating hydrophilic phosphate and hydrophobic selenide groups in its branched backbone, which self-assembles into multi-core/shell micelles composing of many small hydrophobic selenide microdomains which are surrounded by the continuous hydrophilic phosphate phase. Selenium-containing organic molecules usually exhibit sensitive responsiveness to oxidative conditions because of the weak binding energy of carbon-selenium bond.[38] Given the transformation from hydrophobic selenide into the hydrophilic selenone or selenoxide under oxidative stimuli, amphiphilic HPHSeP converts into corresponding hydrophilic species that leads to the disassembly of the micelles (**Figure 5**). The scattered light intensity of HPHSeP micelles decreased dramatically upon the addition of hydrogen peroxide ($H_2O_2$). Furthermore, HPHSeP micelles disassembled into unimolecular micelles after 2 h culture with only 0.1 mM $H_2O_2$ and the selenide groups in HPHSeP were oxidized to selenone other than selenoxide. This system presents the first example of oxidation-triggered disassembly of micelles self-assembled from HBPs.

**Dual reduction and oxidation responsive HBPPs**

Diselenide-containing organic molecules have attracted great attention due to their dual redox-responsiveness.[39] However, the synthesis of diselenide-containing

polymers is still challenging because most of them generally exhibit low solubility and poor stability.[37] Considering the unique characteristics of HBPs, the diselenide-containing HBPs may possess better solubility and stability than that of linear one. In 2012, we first developed a novel approach for the preparation of diselenide-containing organic intermediates[40]. Then the diol made from NaSeSeNa and 2-(2-(2-hydroxyethoxy)ethoxy)ethyl-4-methylbenzenesulfonate was reacted with phosphorus oxychloride resulting in diselenide-containing hyperbranched polyphosphates (HPDSeP). The HPDSeP has alternative diselenide linkages and phosphate groups in the highly branched backbone, which shows good solubility and stability in common solvents, such as dimethylformamide, dimethylsulfoxide, chloroform, dichloromethane etc. Due to the hydrophobicity of diselenide moiety as well as hydrophilicity of phosphate group, HPDSeP can self-assemble into spherical micelles with a multi-core/shell structure in water. HPDSeP was synthesized as the first example of dual reduction and oxidation responsive HBPs, because the diselenide in the backbone are very sensitive to either oxidants or reductants. Se-Se bonds are easily oxidized and broken to seleninic acid under an oxidative condition while reduced to selenol with a reducing agent (**Figure 6**). In the presence of 10 mM glutathione (GSH), the fluorescence emission intensity of Nile red encapsulated HPDSeP micelles declined rapidly within 60 min, confirming the rapid destabilization of the HPDSeP micelles as well as the triggered release of dye molecules. The $^{77}$Se NMR spectrum demonstrated that the diselenide bond was cleaved to selenol by GSH. Similar results were obtained when HPDSeP was treated under oxidation conditions

such as the low concentration of $H_2O_2$ from 0.10 to 1.0 mM.

### 2.3 Modification of HBPPs

Due to a great number of terminal hydroxyl groups, HBPPs can be conveniently modified by different methods. One approach is that various functional compounds are attached to the peripheral terminal hydroxyls of HBPPs via the end-capping reaction, including hydrophilic/hydrophobic, stimuli-responsive, bioactive, and/or therapeutical compounds. Another one is that HBPPs are used as macroinitiators to further initiate the ROP of other different CPMs.

#### End-capping reaction

The terminal modified HBPPs by end-capping reaction were summarized in **Figure 7**. Phospholipids, containing a hydrophilic phosphate or carboxylate ester head-group and two long hydrophobic aliphatic chains, are famous amphiphilic biomolecules with excellent blood compatibility and biocompatibility. A great variety of linear phospholipid analogous polymers have been designed and synthesized for bioapplications.[41] Recently, we developed a novel hyperbranched phospholipid analogous polymers (named HPHEP-alkyls) composing of a hydrophilic HPHEP headgroup and many hydrophobic aliphatic chain tails.[42] HPHEP-alkyls were prepared via reaction of HPHEP with palmitoyl chloride. Due to the amphiphilic nature, HPHEP-alkyls can self-assemble into torispherical micelles in aqueous by the hydrophobic/hydrophilic interactions of alkyl tails and HPHEP headgroups. Through

controlling the fraction of terminal hydroxyl groups capped with hydrophobic palmityls, the micellar size could be adjusted from 98 to 220 nm. Meantime, the critical micelle concentration (CMC) decreases from $1.9\times10^{-2}$ to $3.9\times10^{-3}$ mg $mL^{-1}$ when the grafting ratio increases from 0.63 to 0.90. Ji et al. prepared a series of amphiphilic functionalized HBPPs through modification of hydrophilic HPHEP with hydrophobic 2-diazo-1,2-naphthoquinone (DNQ), spiropyran, or azobenzene etc.[43-45] The obtained amphiphilic hyperbranched multiarm product (HPHEP-DNQ) can self-assemble into micelles with an average diameter of 95 nm in water and a CMC of 25 μg $mL^{-1}$. Since hydrophobic DNQ can be converted to hydrophilic 3-indenecarboxylic acid through the UV-induced Wolff rearrangement, HPHEP-DNQ exhibits amphiphilic to hydrophilic conversion under UV light that induces the disassembly of HPHEP-DNQ micelles. Similarly, amphiphilic HPHEP-spiropyran was synthesized based on the esterification of HPHEP with spiropyran. Different from DNQ, the spiropyran can isomerize to merocyanine under UV light and merocyanine can convert back to spiropyran reversibly when it is exposed under visible light. The diameter of HPHEP-spiropyran micelles decreased gradually after irradiating under UV light and the diameter could be further recovered when the micelles were exposed under visible light. HPHEP-azobenzene was prepared through the carboxylation of hydroxyl groups in HPHEP with succinic anhydride and the subsequent esterification reaction with 4-hydroxyazobenzene. Amphiphilic HPHEP-azobenzene self-assembles into micelles that dissociated gradually upon increasing the amount of *β*-cyclodextrin. Interestingly, the reversible self-assembly and disassembly of HPHEP-azobenzene

can be further realized by irradiating with UV and visible light.

More recently, hydrophilic HPHEP was modified with linear hydrophobic poly(D,L-lactide) by using cucurbit[8]uril as a supramolecular linker (**Figure 8**).[46] In this system, HPHEP and poly(D,L-lactide) were modified with guest molecules of 1-carboxylpropyl-10-methyl-4,4'-bipyridinium and 3-indolepropionic acid, respectively. By the addition of cucurbit[8]uril, a supramolecular linear-hyperbranched amphiphile was formed via the inclusion complexation between cucurbit[8]uril, indole and methyl viologen groups. This amphiphilic polymer can further self-assemble into micelles in aqueous solution. The size of these micelles increased gradually with the addition of a guest molecule of adamantaneamine or a reducing agent of $Na_2S_2O_4$ because of the breakage of the linker and subsequent aggregation of the hydrophobic chains. These dual-responsive micelles show potential applications in drug delivery due to the excellent biocompatibility and biodegradability of both the hyperbranched core and linear arms.

**Macroinitiators**

HBPPs can serve as macroinitiators to further initiate the ROP of other cyclic phosphate monomers to produce copolyphosphates (**Figure 9**). An interesting approach has been developed to synthesize block copolyphosphates with various architectures including linear block, star block and hyperbranched multiarm structures by adjusting the pendent group of CPMs.[34] The hydrophilicity/hydrophobicity of both segments in copolyphosphates is dependent on the side groups. Based on this method,

highly water soluble hyperbranched multiarm copolyphosphates (named as HPHP-*star*-PEPs) were synthesized by using HPHP as macroinitiator and ethyl phospholane (EP) with an ethyl side group as monomer, respectively. Because both the hyperbranched core and linear arms are hydrophilic, so these copolyphosphates are readily soluble in water. Besides, a couple of amphiphilic hyperbranched multiarm copolyphosphates (named as HPHEP-*star*-PIPs) were synthesized by using HPHEP as macroinitiator and isopropyl phospholane (IP) with an isopropyl pendant group as monomer, respectively.[47] HPHEP-*star*-PIPs with a hydrophilic HPHEP core and many hydrophobic linear PIP arms are biocompatible and fully biodegradable. Because of the amphiphilicity, HPHEP-*star*-PIPs can self-assemble into micelles in aqueous media with the diameter from 48 to 74 nm by altering the length of linear hydrophobic PIP arm. In contrast, amphiphilic hyperbranched multiarm copolyphosphates (named as HPHSP-*star*-PEPs) were synthesized by using hydrophobic HPHSP as macroinitiator and EP as monomer, respectively.[48] Similarly, HPHSP-*star*-PEPs can aggregate into spherical micelles in water with a size range from 70 to 200 nm through controlling the length of hydrophilic PEP arms.

## 3 Drug delivery applications of HBPPs

Comparing with linear analogues, HBPs have gained widespread attention for drug delivery applications due to their unique features, such as the relative small hydrodynamic volume for enhanced cellular uptake efficacy, a variety of self-assembled nanostructures with various drug-loading methods, a large population

of functional end groups for further conjugation, etc. However, the biomedical applications of most previous reported HBPs are often limited due to their inherent non-degradability, systemic cytotoxicity, and suboptimal drug release kinetics, etc. Encouragingly, HBPPs have been demonstrated good biodegradability, excellent biocompatibility and low cytotoxicity etc to satisfy these criteria. Four kinds of drug delivery systems based on HBPPs have been developed including non-responsive vehicles, stimuli-responsive vehicles, drug conjugates and self-delivery macromolecular drugs (**Figure 10**).

### 3.1 Biocompatibility and biodegradability of HBPPs

*In vitro* biocompatibility evaluation demonstrates the very low cytotoxicity of HBPPs against different cell lines.[49] Even after incubation with HBPPs up to 10 mg $mL^{-1}$ for 24 h, the viability of treated cells remains nearly 100% compared with that of untreated cells. Moreover, the fluorescence of cells incubated with HBPPs of 10 mg $mL^{-1}$ for 24 h is almost the same as that of the untreated cells according to acridine orange/ethidium bromide staining assays, which further demonstrates the excellent cell compatibility of HBPPs. The *in vitro* degradation study in PBS at 37 °C in different pH value (2.5, 7.4 and 10.5) suggests that HPHEP is degraded through hydrolysis and the basic or acidic environment can accelerate its degradation, especially the acidic one. The degradation of phosphate bond in the backbone of HPHEP reached 50% after ~ 30 days incubation under acidic environment (pH 2.5). This suggests the hydrolysis of phosphate units in HPHEP can be catalyzed by base or

acid. Significantly, cytotoxicity assay suggests the degradation products from HPHEP hydrolyzed at pH 7.4 over 35 days show nearly 100% cell viabilities after 24 h incubation with the concentration up to 10 mg $mL^{-1}$.

### 3.2 Non-responsive carriers

Phospholipid analogous polymers HPHEP-alkyls show great potential for drug delivery because of their excellent biocompatibility. Chlorambucil-loaded HPHEP-alkyl nanocarriers exhibit dose-dependent inhibition and demonstrate the higher inhibition to MCF-7 cell proliferation after 72 h culture in contrast to that of free chlorambucil.[42] The dose of chlorambucil required for 50% cellular growth inhibition ($IC_{50}$) is 5.0 μg $mL^{-1}$ for chlorambucil-loaded HPHEP-alkyl nanocarriers. Conceivably, it is a less efficient way to deliver chlorambucil into the cells in its free form that enters the cell by a concentration gradient. Inversely, it is unidirectional that the chlorambucil-loaded nanocarriers are taken up by the endocytotic system of cells.

Full-polyphosphate nanocarriers were constructed from amphiphilic hyperbranched multiarm copolyphosphates such as HPHEP-*star*-PIPs and HPHSP-*star*-PEPs. These nanocarriers have several potential advantages:[47] (i) the whole micelles are biodegradable and biocompatible. In addition, their low molecular weight degradation products are also nontoxic; (ii) the structures and physicochemical properties of the self-assembled micelles can be controlled flexibly through the facile functionalization of the substituted groups in pentavalent phosphorus; (iii) they have high stability and drug loading capacity in aqueous media. *In vitro* studies suggest that the $IC_{50}$ of

chlorambucil-loaded HPHEP-*star*-PIP nanocarriers against breast MDA-MB-231 cancer cells is 3.0 μg $mL^{-1}$. This *in vitro* anticancer effect might be attributed to the good biodegradability of HPHEP-*star*-PIP nanocarriers that facilitates the drug release from the drug-loaded nanocarriers.

### 3.3 GSH-responsive nanocarriers

Benefiting from the unique self-assembly structure and the existence of many disulfide bonds, HPHSP micelles serving as drug delivery vehicles exhibit several advantages:[36] (i) easy synthesis by mean of "one-pot" preparation; (ii) homogeneous self-assembled micelles with tunable size; (iii) sensitive reduction-responsiveness for site-specific drug release; (iv) excellent biocompatibility and biodegradability for potential clinic applications. *In vitro* cell viability assay data reveal that HPHSP displays no cytotoxicity against NIH 3T3 cells after 48 h incubation with concentration up to 1.0 mg $mL^{-1}$. Drug release results suggest that doxorubicin-loaded (DOX-loaded) HPHSP micelles show controlled release kinetics in respond to DTT and the total DOX release enhances with the increase of DTT from 0.10 to 10 mM (**Figure 11**). Moreover, high intracellular level of GSH accelerates the cleavage of disulfide bond leading to fast DOX release from HPHSP micelles and subsequent rapid localization of DOX in nucleus. *In vitro* anticancer results verify that DOX-loaded HPHSP micelles possess highly efficient anti-proliferation ability.

### 3.4 Reactive oxygen species-responsive (ROS-responsive) nanocarriers

Cancer cells often exhibit elevated intracellular oxidative stress when compared with those of normal counterparts. The intracellular ROS mainly includes $H_2O_2$, hydroxyl radical, and superoxide anions. Considering this exclusive feature of cancer cells, the high intracellular level of ROS can be served as a therapeutic trigger. Drug-loaded HPHSeP micelles were demonstrated to be qualified for ROS-mediated intracellular drug delivery because of the sensitive oxidation-responsiveness.[35] *In vitro* studies of DOX-loaded HPHSeP micelles illustrate that the rapid DOX release can be achieved with the treatment of low concentration of $H_2O_2$ (0.10 mM). Furthermore, the localization of DOX in cell nuclei was found for DOX-loaded HPHSeP micelles after 3 h incubation, indicating the high intracellular level of $H_2O_2$ triggers the destruction of HPHSeP micelles and subsequently induces fast release of the loaded drugs (**Figure 12**). The $IC_{50}$ of loaded-DOX in HPHSeP micelles against Hela cells is only 0.25 μg $mL^{-1}$ after 48 h incubation, which is 10 times lower than that of non-responsive HPHPP micelles under the same conditions. This ROS-mediated drug delivery system based oxidation-responsive HPHSeP micelles show promising applications for cancer therapy.

### 3.5 HBPP-drug conjugates

Prodrugs are some special conjugates composed of hydrophobic anticancer drug and hydrophilic polymer through covalent bond and can be delivered into tumor site more efficient comparing with that of the free drugs. Due to a great number of functional terminal groups in HBPPs, various drug molecules, target groups, and/or fluorescent

agents can be conjugated to achieve multiple functions of treating, targeting, and/or diagnosing, simultaneously. Through esterification of the terminal hydroxyls in hydrophilic HPHEP with the carboxyl in hydrophobic anticancer chlorambucil, amphiphilic HPHEP-chlorambucil conjugates have been synthesized with different drug loading.[49] By adjusting the molar feed ratio of chlorambucil/HPHEP, HPHEP-chlorambucil conjugate with drug loading content up to ~50% was achieved. Meanwhile, the remained hydroxyls can be further used for conjugation of other functional agents, for example, fluorescent rhodamine-B. *In vitro* anticancer evaluation suggests that the $IC_{50}$ value of HPHEP-chlorambucil against MCF-7 breast cancer cells is 75 μg $mL^{-1}$, which is closed to that of free chlorambucil (50 μg $mL^{-1}$). The therapeutic effect may be ascribed to the good biodegradability of HPHEP. The acidity conditions as well as the enzymes in cells accelerate the hydrolysis of phosphate units in HPHEP that results in the continuing release of drugs.

### 3.6 Self-delivery macromolecular drugs

Basically, selenium was considered as an absolute poison in a long time until Schwarz et al identified it as a micronutrient for bacteria, mammals, and birds.[50] Subsequently, organoselenium compounds were found relatively much less toxic compared with the inorganic selenium species. Thus a great number of organoselenium compounds have been investigated as anticancer drugs.[38] However, previous studies are mainly focused on the design and synthesis of small-molecular selenium-containing drugs. Considering the synergistic enhancement of the macromolecular drugs, we speculate

the design and synthesis of hyperbranched macromolecular organoselenium compounds would develop some potent antitumor drugs for cancer therapy.[40] Encouragingly, HPDSeP with many alternative diselenide and phosphate units in the branched backbone exhibits highly efficient anticancer effects in a broad spectrum. The doses of HPDSeP required for $IC_{50}$ for several different human cancer cell lines after 72 h incubation locate in the concentration range between 1.0 and 2.5 mg $mL^{-1}$. However, the analogues of both HPHSP and HPHEP exhibit no therapeutical effects in the identical test conditions of HPDSeP. This suggests the strong anticancer ability of HPDSeP is explained to the diselenide groups distributed along the dendritic backbone. To delivery drugs, especial for hydrophobic anticancer drugs, a drug delivery carrier is required to improve the bioavailability and pharmacokinetics of the drugs. However, the drug carrier itself usually has no any direct therapeutical effect. Therefore, the concerns related to cytotoxicity, degradation and side effects of the carrier materials have been emerged for these drug delivery systems. Differently, HPDSeP can self-assemble into spherical micelles in water with an average size of 50 nm and a critical aggregation concentration of 5.0 mg $L^{-1}$ because of the existence of many hydrophobic diselenide groups as well as hydrophilic phosphate segments in its backbone. This means HPDSeP can spontaneously self-deliver into tumor site via enhanced permeability and retention (EPR) effect (**Figure 13**). Moreover, considering the dual reduction and oxidation responsiveness, HPDSeP micelles can be used as stimuli-responsive drug carriers to encapsulate other hydrophobic anticancer drugs for combination therapy.

## 4 Conclusion and outlook

Topological HBPPs with various characters including hydrophilicity, amphiphilicity, and stimuli-responsive property have been prepared and used to construct advanced drug delivery systems, such as site-specific delivery system, self-delivery system, smart delivery system etc. Although still being at an early stage, HBPPs represent a new kind of important functional polymeric biomaterials and have demonstrated great potential to biological applications. Comparing with their linear analogues, the unique topological structures and properties of HBPPs are in favor of the applications in functional materials and biomedicines that motivate the continuously growing interests in this field.

As an emerging research area, there are many aspects need further investigation or optimization in detail. With respect to chemistry, fundamental aspects between the structure and physicochemical property of HBPPs need to be understood; In regard to functional materials, other kinds of functional HBPPs still need to be explored, such as cationic ones, considering the gene delivery applications. Potential commercial developments should also be promoted. For biomedical applications, the aspects between structure and biocompatibility/biodegradability should be further studied. The fate of HBPPs *in vivo* still remains unknown, especially for drug delivery *in vivo*. Although the researches about HBPPs are going on as well, these unique materials with highly branched architecture and biodegradable backbone are promising for both academic researches and biomedical applications.

**Acknowledgements**

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## Figures

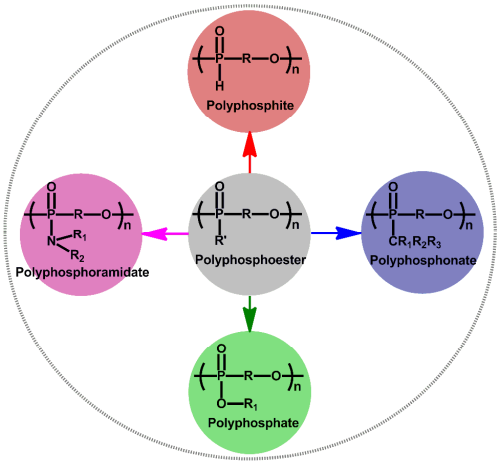



**Figure 1.** Classification of polyphosphoesters based on the side group connected to the phosphorous atom.

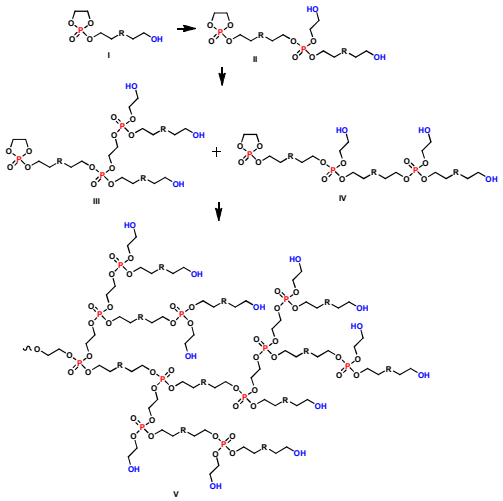


**Figure 2.** Synthesis of HBPPs through SCROP of hydroxyl functionalized cyclic phosphate monomers.

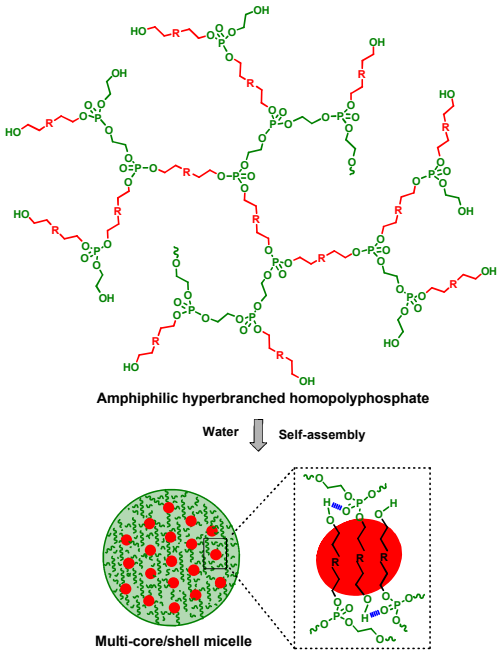



**Figure 3.** Molecular structure of amphiphilic hyperbranched homo-polyphosphates and the illustration of self-assembled multi-core/shell micelles.

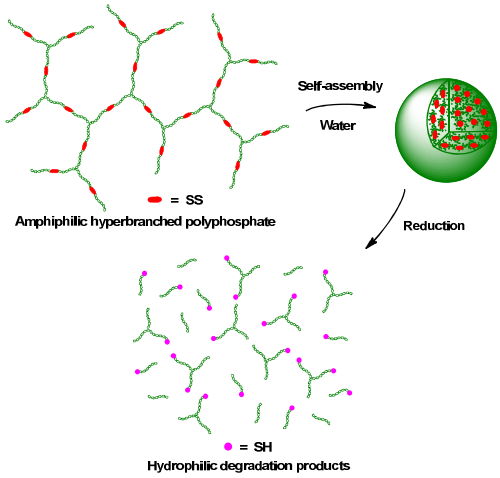



**Figure 4.** Illustration of the self-assembly of HBPPs containing disulfides and the reduction-triggered disassembly of corresponding micelles.

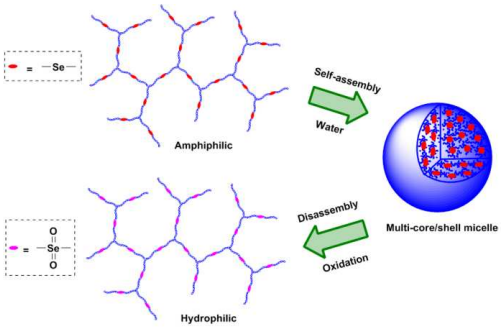



**Figure 5.** The illustration of the selenide-containing HBPPs self-assembly and the oxidation-triggered disassembly of corresponding micelles.

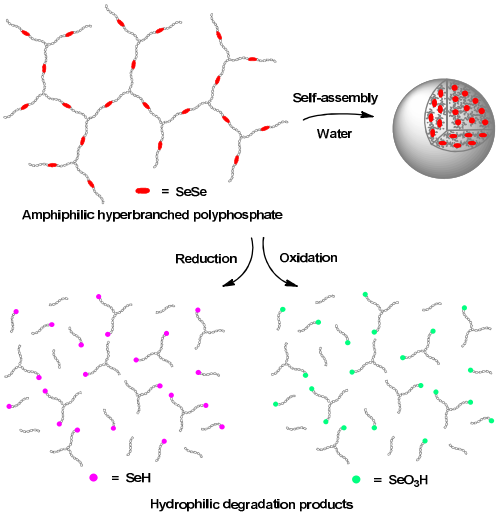



**Figure 6.** Illustration of diselenide-containing HBPPs self-assembly and the dual reduction and oxidation triggered disassembly of corresponding micelles.

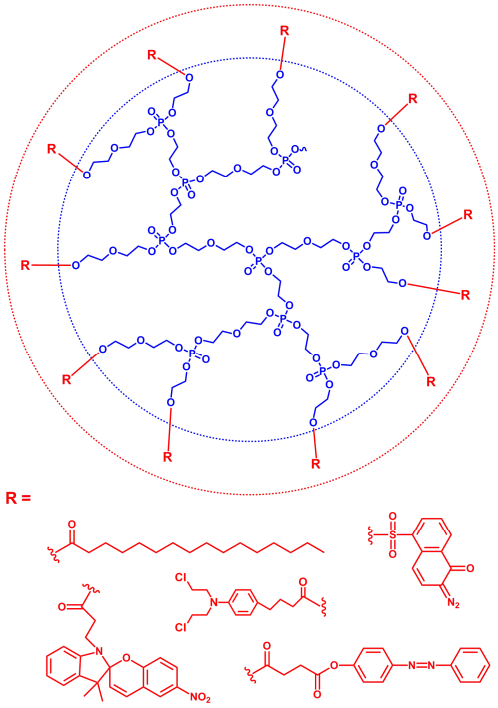



**Figure 7.** Modification of hydrophilic HBPPs with various hydrophobic functional molecules by the end-capping reaction.

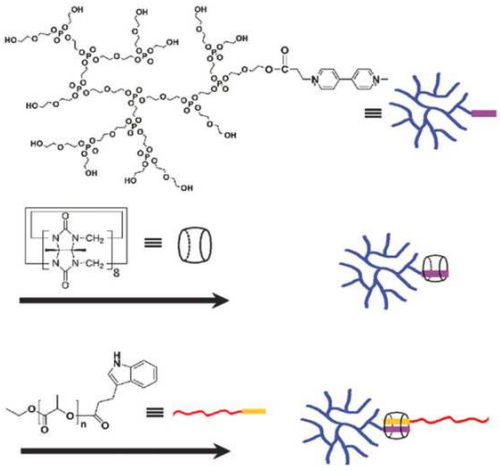



**Figure 8.** Modification of HBPPs based on a supramolecular linker. Reproduced with permission from ref. 48. Copyright 2013, Royal Society of Chemistry.

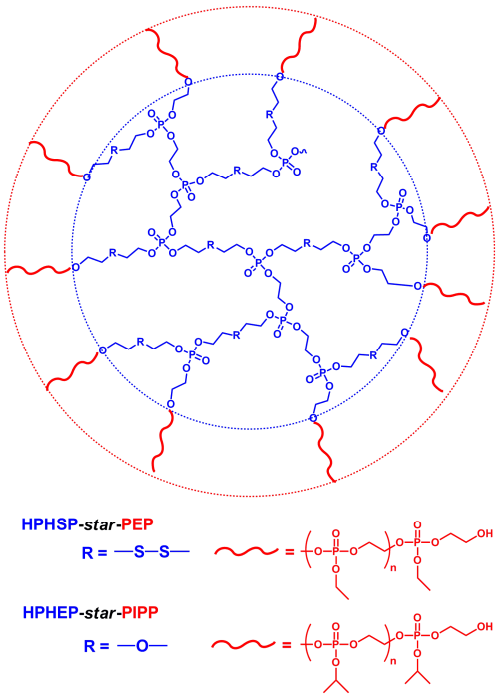



**Figure 9.** Hyperbranched multiarm copolyphosphates synthesized by using HBPPs as macroinitiators.

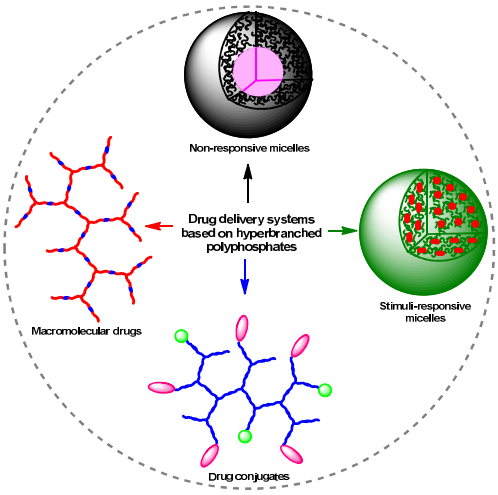



**Figure 10.** Classification of drug carriers based on HBPPs.

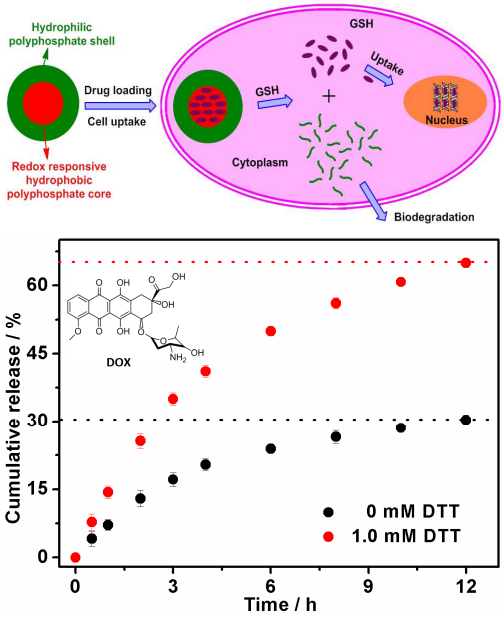



**Figure 11.** Illustration of reduction-responsive HPHSP-*star*-PEP micelles for intracellular drug release triggered by GSH (above) and *in vitro* release of DOX from HPHSP-*star*-PEP micelles with or without treatment of DTT. Reproduced with permission from ref. 50. Copyright 2011, American Chemical Society.

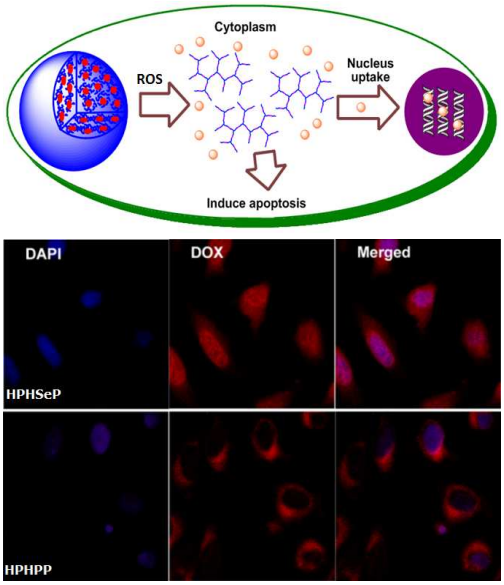



**Figure 12.** Oxidation-responsive HPHSeP micelles rapidly release the loaded drugs triggered by the ROS in cancer cells (above) and confocal images of Hela cells incubated with DOX-loaded HPHSeP micelles and HPHPP micelles for 3.0 h (below). Cell nuclei were stained by DAPI. Reproduced with permission from ref. 37. Copyright 2013, American Chemical Society.

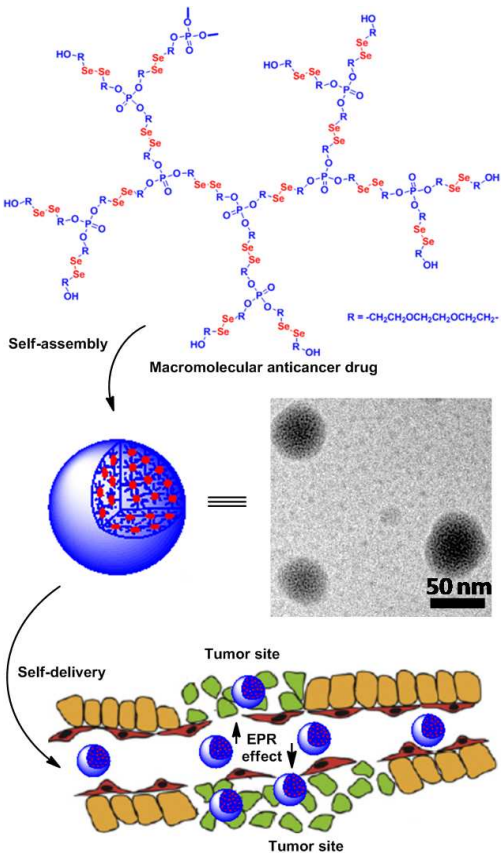
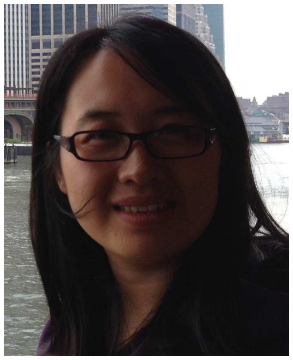



**Figure 13.** Illustration for self-delivery of macromolecular anticancer drugs. Reproduced with permission from ref. 42. Copyright 2012, Elsevier.

Jinyao Liu received his PhD degree in Materials Science in 2013 from Shanghai Jiao Tong University where he worked under the supervision of Prof. Deyue Yan on the syntheses and drug delivery applications of hyperbranched polyphosphates. He is currently working as a postdoctoral associate in the research group of Prof. Ashutosh Chilkoti at Duke University and will move to Prof. Robert Langer's group at MIT. His research focuses on the development of strategies to synthesize well-defined polymeric therapeutics for the treatment of various diseases.



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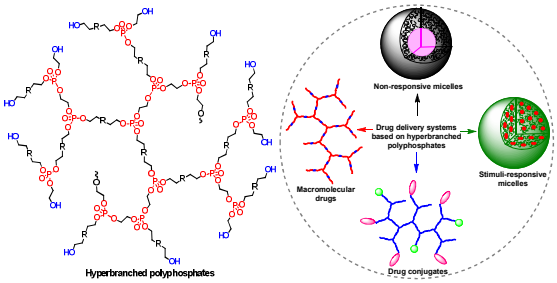
Deyue Yan received his BS degree in Chemistry at Nankai University and MS degree in Polymer Chemistry at Jilin University. In 2002, he obtained his PhD degree in Polymer Chemistry at Catholic University of Leuven, Belgium. He began his work at East China University of Science and Technology as a lecturer in 1966, and then moved to Tongji University in 1980 as an associate professor. From 1987 he is a full professor at Shanghai Jiao Tong University. His main research interest concerns polymerization kinetics theory, the synthesis and application of hyperbranched polymers, the modification of carbon nanotubes, supramolecular self-assembly, etc.

**Table of contents**




This review covers the recent development of hyperbranched polyphosphates, including synthesis, functionalization and biomedical applications that may promote the interdiscipline researches among dendritic polymer, functional material, and biomedicine.